A STUDY OF THE DIASTATIC INDEX OF THE URINE IN EPIDEMIC PAROTITIS AND IN THE NORMAL SUBJECT.

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In the course of general practice, when attending a case of Epidemic Parotitis in a child, one was struck by a strong odour of acetone in the breath of the patient. On enquiry of the mother, one elicited the fact that there had been vomiting, unrelated to the taking of food, both on that, and on the previous day. The urine was tested for sugar by Fehling's and Benedict's methods, but gave a negative result. The ferric chloride test was positive, as was the nitro-prusside test for acetone. The vomiting had not been so severe as to cause such a degree of ketosis, and a rectal glucose administration with a small dose (10 units) of insulin was suffucient to cure the condition.

As this occurred on the second day of the disease, it seemed very doubtful that pancreatitis had occurred as a complication of the parotitis, the experience of C.B.Ker being for this to occur later. He does, however, quote a case in which the pancreat--itis appeared to precede the parotid swelling. It appeared more likely that the child was a latent case of diabetes mellitus, stimulated into activity by the supervening infection, the absence of sugar from the urine being accounted for by a high renal threshold coupled with two days or so of almost total abstinence from food. As the child was easily frightened, the parents likewise, a blood sugar estimation was postponed pending a test for pancreatitis on the urine.

Starch digestion was found to be greatly increased above the normal, the reading being over 100 Wohlgemuth units. As the child was now much improved generally, no further steps were taken beyond the ordinary treatment of mumps.

Four days after this patient's illness commenced, her sister developed stiffness of the neck followed by a parotid swelling on one side. Her urine also gave a high reading, the diastase content being above 50 units. In this case there was no acetone in the breath or urine.

From the findings in these two cases it appeared to be possible either that pancreatitis of a certain degree was more frequent during mumps than the records of cases suggest, or that the diastase in the urine was derived from the parotids themselves. If the former supposition were correct, then mumps might be utilised for a study of pancreatic dysfunction, about which, in mild degrees, there is little published work; if the latter, then study of other cases of epidemic parotitis might result in the development of an easily performed diagnostic test, which would have useful application in those cases which occur at the commencement of an epidemic, in which, perhaps there is only one swollen gland, and the diagnosis,

apart from clinical observation, can only rest upon a white blood cell count; a leucocytosis being in favour of abscess or suppuration, and a normal count or a leucopenia being suggestive, but not diagnostic of epidemic parotitis.

Specimens of urine, therefore, were obtained from a number of cases of undoubted mumps, tested for acetone, and the diastase content estimated at various periods during the course of the disease.

At the same time those cases occurring in one's own practice were tested by Loewi's adrenalin reaction for pancreatitis.

Since it was desirable to estimate the diastase content as accurately as possible, and as Wohlgemuth's geometric progressions become too widely spaced in the higher titres to give more than an approximate idea of the amount of diastase present, a modified technique was devised whereby the steps become more regular and more closely spaced.

The method used is as follows;-

The urine is diluted 1/7 using as diluent a phosphate buffer solution, the final pH being adjusted to 6.1. The presence of chlorides in the urines was not taken into account.

Fifteentubes 1/2 x 4" are set up in a rack and measured quantities of diluted urine are pipetted to the bottom of the tubes as shown in Table A. To keep the volume constant, the bulk was made up to 1 cc with distilled water.

TABLE A.

Number of tube	Urine (1/7) cc	Dist. Water cc
1	1.0	0.0
2	0.9	0.1
3	0.8	0.2
4	0.7	0.3
5	0.6	0.4
6	0.5	0.5
7	0.4	0.6
8	0.35	0.65
9	0.3	0.7
10	0.25	0.75
11	0.2	0.8
12	0.15	0.85
13	0.1	0.9
14	0.05	0.95
15	0.0	1.0

Tests were subsequently controlled to find whether the addition of distilled water made any difference to the final reading. No difference was noted in ten cases, so the water was omitted. This applies only where high readings may be expected.

2.0 cc of 0.1% starch solution, using Pulv. Amyli of the B.P. were then added as

rapidly as possible to each tube, and the rack containing the tubes placed in the water bath at 37 C. for exactly half an hour, each tube being first inverted to ensure mixing.

On removing from the water bath, the tubes were rapidly filled up with cold distilled water to slow down the rate of digestion as rapidly as possible, and asnearly as possible, simultaneously in all tubes. One drop of N/20 iodine in aqueous solution was then delivered from a capillary pipette to each tube, the tubes were again inverted, and readings made.

Two or three tubes usually show the pink tint due to the presence of erythrodextrin, fading off into mauve and purple. Taking the last tube which shows no colour whatever as the end - point gives a conservative estimate of the diastase present, and that has been done in this series. If the tubes are allowed to stand at room temperature or in the cold overnight, the pink colour fades, the tubes becoming colourless, while the blue or purple is maintained, making the end-point clear and sharp. This point is rather too high.

Up to date, twenty - two cases of epidemic parotitis have been examined at various times during the course of the disease and in convalescence. All unquestionable cases gave a distinctly positive result, while one adult, (case XV) who was under the care of another practitioner gave a reading within the normal limits. This case, however, was not noticed until late in the disease, and as only one gland had been swollen, the diagnosis is open to doubt.

IN reckoning the day of the disease the first day has been assumed to be that day on which there was sore throat, stiffness of the neck and a feeling of malaise, but probably no parotid swelling, or at any rate, no very pronounced swelling

Tests were also carried out on other patients with a variety of conditions which, unfortunately, do not constitute good controls. The reason for the inclusion among the control cases of such a condition as influenza is as follows; -

The amount of urinary diastase might be materially influenced by the needs of the body for the ferment for digestive purposes, surplus ferment being excreted in the urine. The anorexia associated with any fever, might account

for some increase in the excretion of diastase, assuming this supposition to be correct.

Therefore cases of influenza, in which there was complete loss of appetite were selected to reinforce the normal healthy controls which are referred to later.

However, there are among these controls three cases which were not mumps, but had swelling of one or both parotids due to other causes.

Details of the cases of epidemic parotitis are tabulated in Table B., and expressed more fully graphically.

TABLE B.

No. of case	Glands One or both	Acetone	No. of tests	Average units D. 14 days.
1	1	++	4	78
2	2	-	1	45
3	2	++	4	50
4	2	-	4	82
5			1	-
6	2	+	4	83
7	2	+	2	55
8	2	-	3	46
9	2	-	3	71
10	2	+	4	115
11	2	-	2	40
12	1	-	2	48
13	2	tr	3	160
14	2	-	5	89
15	1	-	2	19
16	1	-	3	27
17	2	tr	6	77
18	1	-	1	40
19	2	-	3	82
20	2	+	5	105
21	2	-	7	98
22	2	-	5	92

See also charts I - XXII.

Amongst the control cases are three instances of parotid swelling, one due to abscess of the gland, one due to the reaction caused by erysipelas of the face, and one case of obscure gerealised infection with pyrexia, splenic enlargement and bilateral induration and swelling of the parotids which had been present for some weeks. The saliva of this last patient, which was scanty, was found to possess no amylolytic ferment, and it appeared likely that there might be absorption into the blood. The

The urine, however, had a normal content of diastase (12 units) from which one assumes that the parotid function was either abolished or in abeyance. The other control cases except the case of erysipelas of the face gave results well within the normal limits. These results are tabulated below;-

TABLE C.

No. of case	Average conc. D.	No. of tests.	Notes
1	6	2	Acute gastritis
2	12	4	acute nephritis
3	40	2	Erysipelas of face
4	18	1	Influenza
5	18	1	Influenza
6	16	1	Influenza
7	18	2	Pneumonia
8	8	1	Abscess of parotid
9	27	3	Carcinoma of pancreas
10	16	2	Parotitis after tonsilitis
11	12	1	Parotid induration with septicaemia.

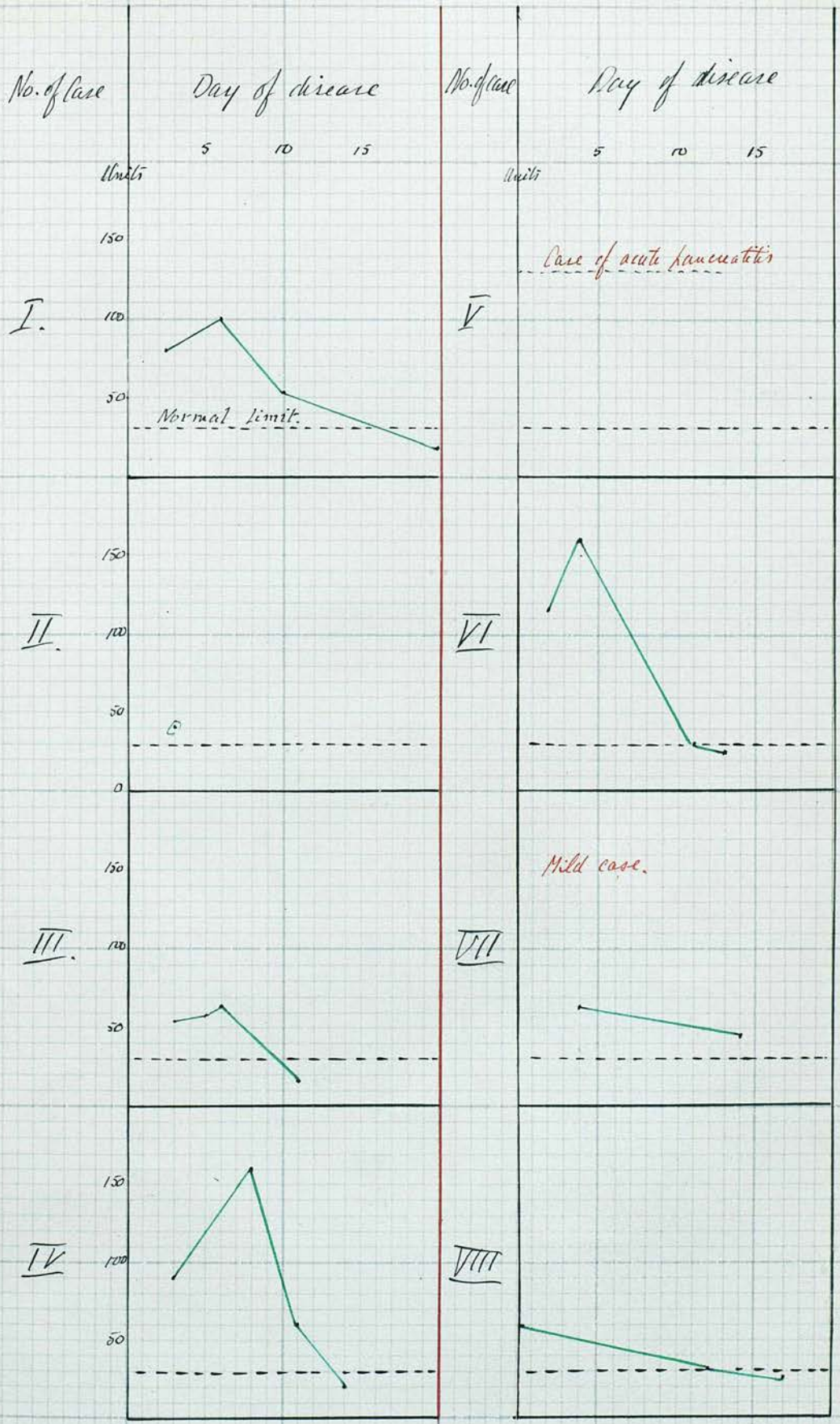
Beyond adjusting the urines tested to an approximately optimum pH no precautions were taken to secure a uniform excretion rate, or by dilution according to the method of Stafford and Addis (2), to compensate for the variations of rate. Indeed the exigencies of practice would not permit of this, for none of these cases were in hospital. This failure to secure uniformity in this respect no

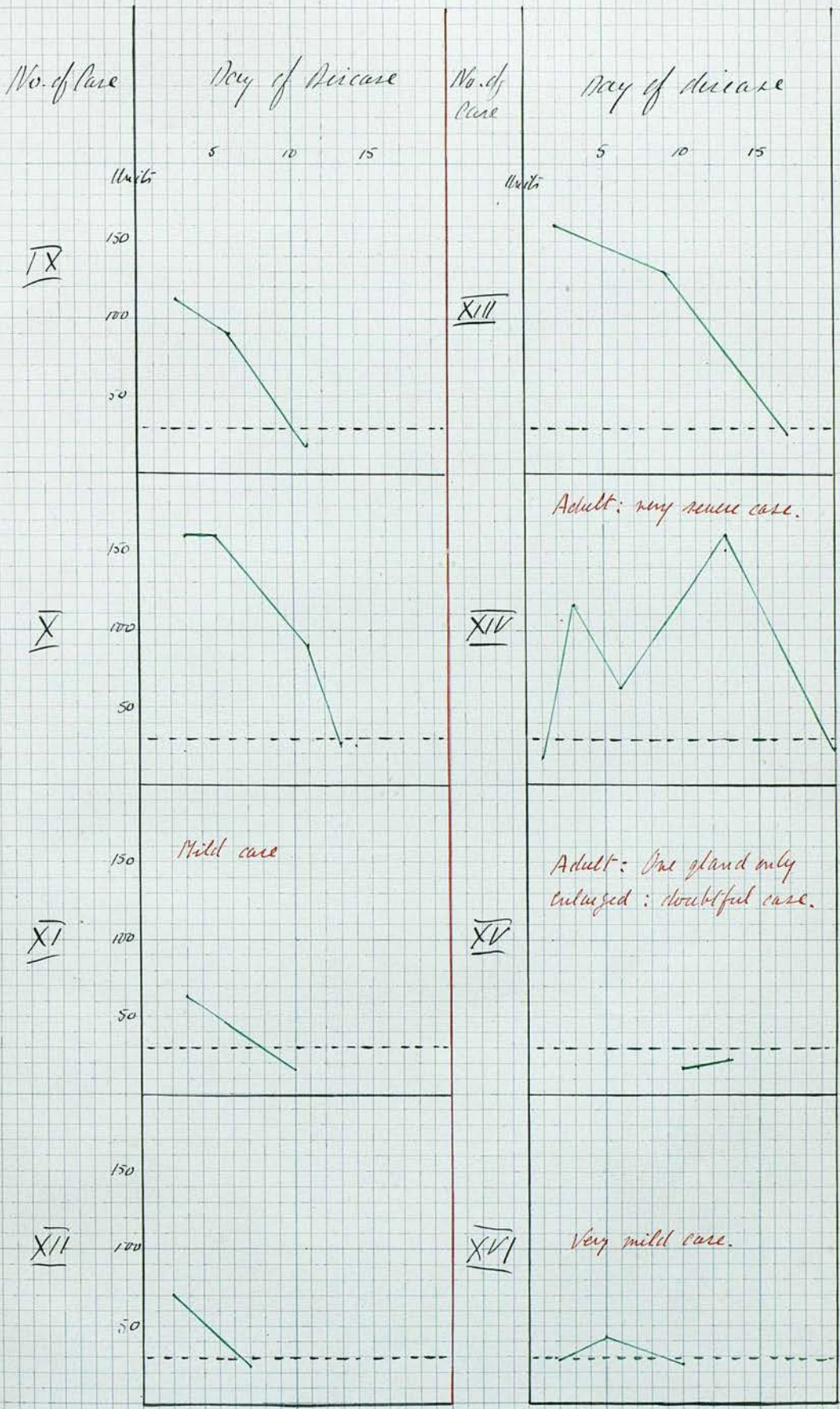
doubt accounts, in great measure for the lack of maintenance of level in some of the graphs. But where the diastase has been so greatly increased above the normal, this apparent variation has not been sufficient to obscure the issue that the diastatic index of the urine in mumps is raised in a degree corresponding to that prevailing in acute pancreatitis.

Each case is detailed in the graphs.

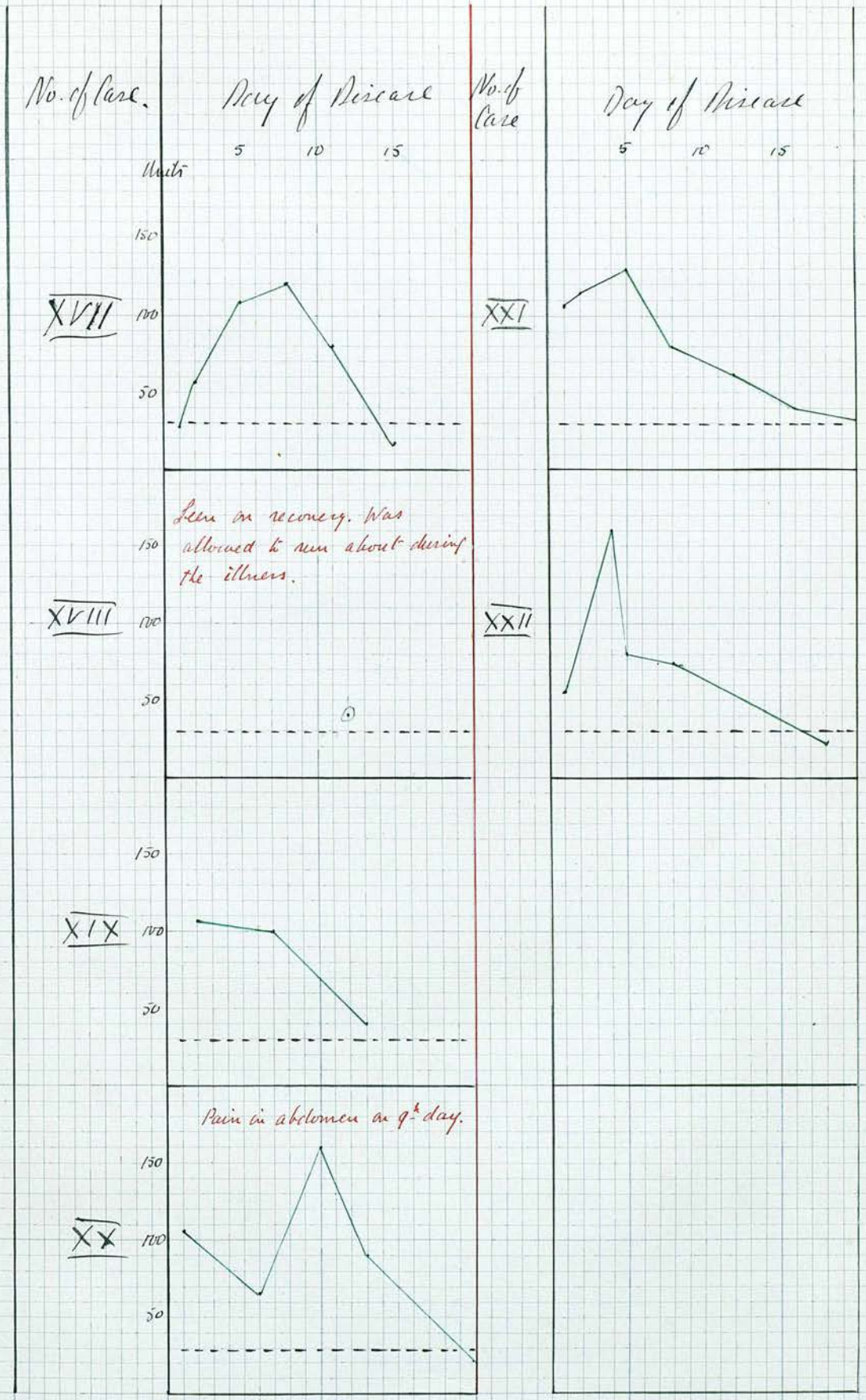
As many authorities are agreed that the upper limit of normality is in the region of 30 units of diastase, this has been marked in. The green line represents the level of diastase measured in Wohlgemuth units, as it was found on various days during the course of the disease.

To return for a moment to the question of normal variation; Stafford and Addis state that the conecentration of diastase per cc. can be confudently expected to vary in inverse ratio to the volume of urine per hour, and, further, that the excretion rate of diastase in units per hour remains fairly constant inspite of marked variation in the output of urine. These workers point out that most results are given in terms of concentration of diastase, and insist upon the ∅ desirability of referring to the rate of excretion instead.


No. of case
Day of disease
5
10
15
Units
150
100
50
0
I.
Normal limit.
II.
III.
IV
V
Case of acute pancreatitis
VI
VII
Mild case.
VIII


No. of Case
Day of Disease
5
10
15
Units
150
100
50
IX
X
XI
XII
Mild case
No. of Case
Day of Disease
5
10
15
Units
XIII
Adult: very severe case.
XIV
Adult: One gland only enlarged: doubtful case.
XV
Very mild case.
XVI

No. of Case. | Day of Disease | No. of Case | Day of Disease



In the case of an acute febrile illness, therefore, in which there is but scanty secretion of urine, the concentration of diastase per cc. must be expected to be high. That it is higher in mumps than may be accounted for by this factor is shown by the work of these authors and by Reid (5) on normal subjects in conditions under which the output of urine is exceptionally low. 33.5 units per cc. appears to be the upper limit of normal excretion.

Twenty one cases of mumps were tested repeatedly by Loewi's method of instillation of 1/1000 adrenalin solution into the conjunctival sac for evidence of pancreatitis. No positive reactions were observed. If this be accepted as evidence of absence of pancreatitis, then the increased output of diastase in the urine can only be due to the parotid infection. It was impossible owing to the circumstances in which these cases were attended to test for evidence in the faeces of lack of pancreatic activity.

The epidemic of parotitis having subsided an attempt has been made to determine, if possible, any factors influencing the normal excretion of diastase. For this purpose, similar technique to that used for

TABLE ~~II~~ D.

Date	Time	S.G.	Vol.	Mins. Output per min.	Conc. D. per cc.	Units D. per min.	Notes.
2-3-'32	7.30 p.m. to 11.0 p.m.	1020	270 cc.	1.3 cc.	15.3	21.9	Meal 8.0 p.m. Beer 500 cc.
"	11.30 p.m.	1024	35 cc	1.7 cc	27.5	46.7	" "
3-3-'32	9.0 a.m.	1030	210 cc	0.37 cc	30+	11.1	Meal 8.0 p.m. on 2-3-'32.
"	9.50 a.m.	1024	40 cc	0.8 cc	27.5	22.0	Meal 9.0 a.m.
"	5.0 p.m. to 5.35 p.m.	1016	77 cc	2.2 cc	12.5	27.5	Meal 4.30. Carbohydrates.
"	7.0 p.m.	1020	94 cc	0.8 cc	27.5	22.0	"
"	8.0 p.m.	1022	60 cc	1.0 cc	27.5+	27.5	Meal 7.30. Gin 3j
"	11.15 p.m.	1012	330 cc	1.7 cc	27.5+	46.8	Tea 1 cup 10.30 p.m.
4-3-'32	1.35 p.m. to 2.45 p.m.	1026	75 cc	1.1 cc	15.3	16.4	Beer 250 cc at 12.0 noon lunch 1.30 p.m.
"	4.10 p.m.	1024	100 cc	1.2 cc	14.2	17.0	"
"	4.50 p.m.	1022	45 cc	1.1 cc	15.3	16.4	Tea at 4.0 p.m. Carbohydrates.
"	8.0 p.m. to 9.30 p.m.	1020	35 cc	0.39 cc	15.3	5.97	Meal at 8.0. Proteins.
"	11.15 p.m.	1028	92 cc	0.88 cc	15.3	13.5	"
5-3-'32	5.30 a.m.	10.31	133 cc	0.35 cc	20.0	7.0	"
"	7.0 a.m.	1032	40 cc	0.4 cc	20.0	8.0	Cup of tea at 6.0 a.m.
6-3-'32	5.15 p.m. to 6.45 p.m.	1005	244 cc	3.3 cc	6.0	19.8	Tea 4.30 p.m.
"	7.30 p.m.	1012	82 cc	1.82 cc	6.6	11.9	"
"	8.30 p.m.	1014	70 cc	1.15 cc	6.6	7.6	Beer 1 pint at 7.45 p.m.
"	10.55 p.m.	1016	202 cc	1.4 cc	6.6	9.24	"
8-3-'32	10.55 a.m. to 12.5 p.m.	1022	90 cc	1.28 cc	12.5	16.0	Meal at 9.0 a.m.
"	1.35 p.m.	1026	107 cc	1.19 cc	15.3	18.2	Beer 500 cc. at 12.30 p.m.
"	5.25 p.m.	1020	134 cc	0.58 cc	12.5	7.25	Tea at 5.0 p.m.
9-3-'32	11.20 p.m. to 1.25 a.m.	1018	175 cc	1.4 cc	10.5	14.7	Cup tea at 10.30 p.m.
"	9.40 a.m.	1030	188 cc	0.38 cc	20.0	7.6	"

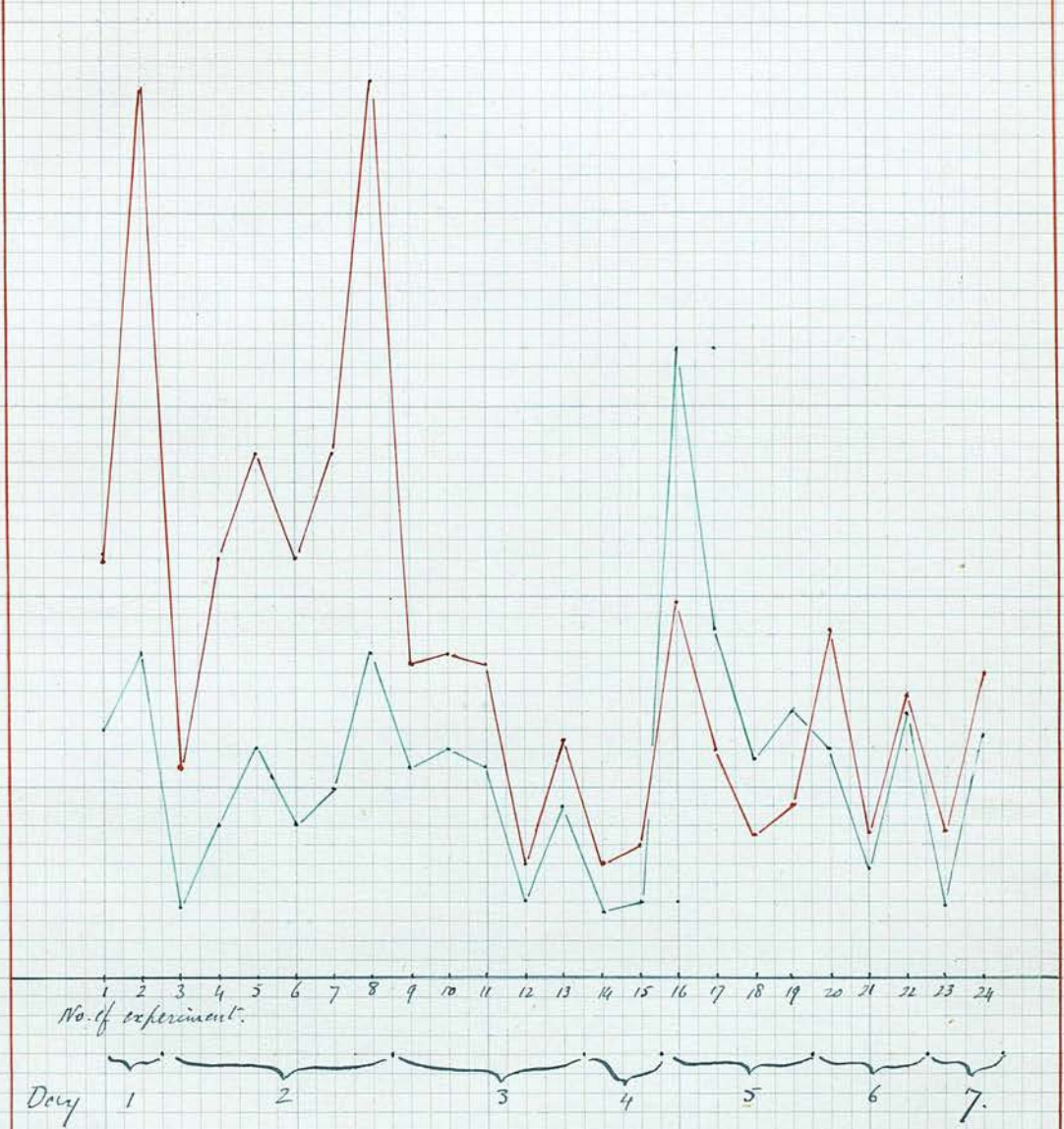
cases of parotitis was employed, but the urine was diluted using equal parts of urine and buffer solution. The amount pipetted to the fifteen tubes were modified to give readings ranging from 6 to 30 units of diastase.

While it appears that the variation in the concentration of diastase varies inversely as the output of urine, it does not vary proportionately, the ratios being widely different. But when the excretion rate of urine per minute is compared with that of diastase it is seen that there is fairly close general relation-ship, which is most pronounced during the normal hours of sleep. Plotting a curve of these two factors shows that during the day the curve of diastase swings away from that of urinary output, but always in the same direction. See Table D. and Chart XXIII.

This, admittedly, does not agree with the observations of Stafford and Addis who found that the diastase output per hour remained at about the same level in spite of pronounced changes in the rate of output of urine. In their experiments, however, the changes in urinary output were deliberately produc-ed by the drinking of quantities of water, and one contends that such conditions are not those that normally obtain, and that such observations , as

CHART XXIII

Chart to Show Relationship Between
Excretion Rate of Diastase per minute ——
Output of Urine per minute ——



There is no continuity between one day and the next; the apparently anomalous result between days 5 and 6 is due to this lack of continuity.

indices of normality should be accepted with reserve. It is obviously undesirable, if a variation from the normal in pathological conditions is to be looked for, that the standard of comparison should be one which is unnatural. If such is to accepted as the standard, then to make comparison, similar unnatural conditions must be established for the pathological subject; and to superimpose unnatural conditions upon an already obscure state is to render the problem more difficult than it already is.

Consequently, in working with specimens taken from a normal person, no attempt has been made to create conditions, but the bladder was emptied frequently and the conditions obtaining at the time of micturition noted.

At first it appeared that there was some relation between the specific gravity of the urine and the concentration of diastase, but this was quickly upset by further observations. The effect of diet also appeared to be quite uncertain, carbohydrate meals, calling, one might expect, for use of all the amylolytic reserves of the body, do not regularly diminish the output, nor is there any increase to be obtained following the ingestion of malted liquors or even of preparations having a specially high diastase

content.

The ingestion of carbohydrate meals may be objected to on the grounds that an enzyme is not used up by its own activity, and that a limited quantity of diastase in the body will be capable of dealing with an unlimited amount of starch; as it is; but on the other hand, normal digestion proceeds at a certain rate, and if an unusual amount of starch is to be dealt with in a given time, an unusual amount of diastase will be required to execute the work. (cf. the peaked curve found in normal subjects when the sugar tolerance is estimated by means of starch administration).

To carry the argument further, if the above supposition is correct, then the work being done, there should ensue a large increase in the amount of surplus diastase to be excreted in the urine. These experiments show no evidence of any such occurrence. Even when no food was taken from 9.0 a.m. until a large meal of carbo-hydrate at 4.30 p.m., the diastase output at 5.35 and 7.0 p.m. showed no great variation from that ocurring at 9.50 a.m. on the same day. On a subsequent occasion when the process was repeated, the diastase output fell slightly.

C. Reid (5) agrees with Stafford and Addis that the excretion rate of diastase per hour varies with the output of urine in inverse ratio. He qualifies this by remarking that "exceptions were rather frequent". In both these papers the figures represent rates per hour. But in the course of one hour the output of water can vary by as much as 200% or even more, while the diastase excretion is liable to equally great variation which, moreover, is by no means parallel. One may say therefore, that there are in the course of one hour perhaps ten possible variations; and since the most efficient urinary apparatus cannot produce urine oftener than four times in the hour under normal conditions , the ideal method of securing specimens would be by means of ureteral catheters, examining a specimen every five minutes or so. This is, of course, impracticable in ordinary circumstances. But if the bladder is emptied as frequently as possible, and the results reduced to excretion rate per minute, the fallacies are reduced to a minimum. This is mere

--------- This is parenthesis; but returning to Reid's somewhat hesitant statement of the relation between water elimination and diastase excretion, his figures in his Table V show the opposite when the rate of urine output is compared with the average hourly diastatic activity.

Reid's Table V (abstract)

Rate of urine secretion per hour in cc.		Average hourly total diastatic activity.	
33	~~increase~~	528	
36	increase	720	increase
58	"	932	"
94	"	1132	"
87	decrease	883	decrease
67	"	674	"
33	"	532	"
27	"	486	"

Unfortunately there is a mistake in the second figure, which should read 72. More unfortunately still this is the only exception to the rule one is endeavouring to establish.

It had been hoped to carry out some tests showing the effects of physiological substances such as adrenalin on the excretion of diastase. Time, however, has precluded this work at present. Reid has found thatexcitement causes a pronounced diminution in the rate of excretion.

In the above series of tests on the normal, there has been made one assumption which may be erroneous. One has assumed that one's own kidneys are efficient enough to be regarded as a control for the diastase test. There is no sign of dysfunction (e.g. orthostatic albuminuria) and one is always

more or less in good condition from the atletic point of view, and capable of standing an average amount of fatigue. It does appear , however, that there is a certain reluctance to produce a diuresis compared with Addis' normal case, and perhaps there is no real justification for some of the conclusions arrived at in the study of the normal.

CONCLUSIONS.

1. In cases of epidemic parotitis the urinary diasta-ase is increased to an extent commensurate with that found in cases of acute pancreatitis. This increase occures early in the disease, and persists until convalescence.

2. There is no apparent increase in the diastase content of the urine in cases of parotid swelling not due to mumps.

3. This rise in the diastase content is so marked, and so constant as to be of service as a diagnostic test where there is doubt as to the nature of the swelling.

4. Twenty one cases of mumps were tested by Loewi's method for pancreatitis, but all showed a negative re-action. Thus it appears unlikely that the high diast-ase content of the urine in mumps is due to coexistent

inflammation of the pancreas.

5. In the normal subject, diet or the taking of fluids has little or no influence on the rate of excretion of diastase by the kidneys; and provided the rate of excretion is measured, and not merely the concentration, no special precautions need be taken before making estimations.

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